

201-16019A

**HIGH PRODUCTION VOLUME (HPV)  
CHEMICAL CHALLENGE PROGRAM**

05 SEP -2 AM 9:03

RECEIVED  
AMPT/MLD

**TEST PLAN  
for  
Phosphonic acid, (2-chloroethyl)-, bis(2-chloroethyl) ester  
[BISCEP Monomer]  
CAS No. 6294-34-4**

**and**

**Phosphonic acid, [2-[[[(2-chloroethoxy)(2-chloroethyl)  
phosphinyl]oxy]ethyl]-, bis(2-chloroethyl) ester  
[BISCEP Dimer]  
CAS No. 58823-09-9**

**Submitted to the US EPA  
by  
Rhodia Inc.**

**August 24, 2005**

## I) INTRODUCTION

Under the U.S. Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program, Rhodia Inc. (formerly Albright & Wilson Americas Inc.) voluntarily committed to compile basic screening data on:

- Phosphonic Acid, (2-chloroethyl)-, bis(2-chloroethyl) ester  
CAS No. 6294-34-4
- Phosphonic Acid, [2-[[[(2-chloroethoxy)(2-chloroethyl)phosphinyloxy]ethyl]-, bis(2-chloroethyl) ester  
CAS No. 58823-09-9

The material, known as BISCEP, is produced as a mixture of the monomer (CAS No. 6294-34-4) and dimer (CAS No. 58823-09-9). BISCEP contains ca. 55-70% w/w BISCEP monomer and ca. 35-40% w/w BISCEP dimer. Because of this, the existing test data discussed in this test plan have been primarily conducted on this BISCEP mixture and thus the monomer and dimer are addressed and reported in the same test plan. For the remainder of this dossier, we will refer to BISCEP, recognizing we are designating the monomer-dimer mixture.

This test plan follows up on that commitment. Specifically, this test plan sets forth how Rhodia intends to address testing information for BISCEP.

In preparing this test plan the following steps were undertaken:

Step 1: A search was conducted for relevant published and unpublished literature on BISCEP.

Step 2: The compiled data was evaluated for adequacy in accordance with the EPA guidance documentation.

## II) GENERAL SUBSTANCE INFORMATION

Chemical Name: **Phosphonic acid, (2-chloroethyl)-, bis(2-chloroethyl) ester**

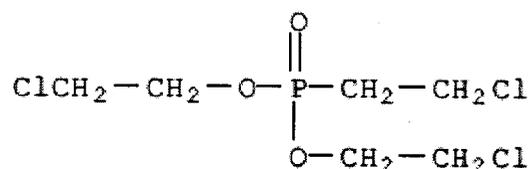
Chemical Abstract Service Registry Number: CAS No.: 6294-34-4

Synonyms: BISCEP Monomer  
Bis(2-chloroethyl) 2-chloroethylphosphonate  
Bis(2-chloroethyl) (2-chloroethyl)phosphonate  
Bis-chloroethyl 2-chloroethanephosphonate

Molecular Formula:  $C_6H_{12}Cl_3O_3P$

Molecular Weight: 269.7 g/mol

Structural Diagram:



Phosphonic acid, (2-chloroethyl)-, bis(2-chloroethyl) ester  
CAS No. 6294-34-4

Chemical Name: **Phosphonic acid, [2-[[[(2-chloroethoxy)(2-chloroethyl) phosphinyl]oxy]ethyl]-, bis(2-chloroethyl) ester**

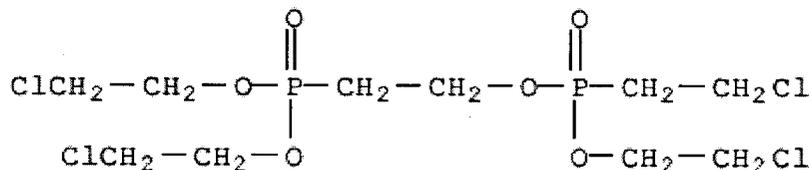
Chemical Abstract Service Registry Number: CAS No.: 58823-09-9

Synonyms: BISCEP Dimer  
Bis(2-chloroethyl) [2-[[[(2-chloroethoxy)(2-chloroethyl)phosphinyl]oxy]ethyl]phosphonate  
[2-[[[(2-Chloroethoxy)(2-chloroethyl)phosphinyl]oxy]ethyl]phosphonic acid, bis(2-chloroethyl) ester

Molecular Formula: C<sub>10</sub>H<sub>20</sub>Cl<sub>4</sub>O<sub>6</sub>P<sub>2</sub>

Molecular Weight: 440.2 g/mol

Structural Diagram:



Phosphonic acid, [2-[[[(2-chloroethoxy)(2-chloroethyl) phosphinyl]oxy]ethyl]-, bis(2-chloroethyl) ester  
CAS No. 58823-09-9

### III) USE AND EXPOSURE INFORMATION

The subject mixture, internally known as BISCEP, consists of the BISCEP monomer (CAS No. 6294-34-4) and BISCEP dimer (CAS No. 58823-09-9). This mixture is manufactured by a single US producer, Rhodia Inc., at a single manufacturing site. BISCEP is used primarily as an internal chemical intermediate to produce a crop protection active ingredient (plant growth regulator) at the same production facility. An extensive EPA database exists on this downstream product -- phosphonic acid, 2-chloroethyl-. There is minimal opportunity for occupational exposure during the manufacture of either BISCEP or the downstream product. In addition, a small amount of BISCEP is commercially sold as a flame retardant. Again, there is limited opportunity for exposure during its use in this application.

### IV) REVIEW OF EXISTING DATA AND DEVELOPMENT OF TEST PLAN

Rhodia Inc. has undertaken a comprehensive evaluation of all relevant data on the SIDS endpoints of concern for BISCEP.

The more significant and reliable data are gathered in table 1. The availability of the data on the specific SIDS endpoints is summarized in table 2.

### **1) Review of existing physicochemical data and proposed testing**

Data are available in Rhodia MSDS or in Handbook for melting point, boiling point, density and water solubility. Vapor pressure and octanol/water partition coefficient were estimated with the EPIWIN program. Estimations were performed on BISCEP monomer (CAS No 6294-34-4, the main component of BISCEP) and BISCEP dimer (CAS No 58823-09-9, the other main component of BISCEP (noted as main impurity in IUCLID as IUCLID was designed to address substances rather than mixtures)). Physico-chemical properties needed individually on BISCEP monomer and BISCEP dimer for the fugacity level III modeling were also estimated with the EPIWIN program.

No additional testing is proposed for purposes of the HPV program.

### **2) Review of existing environmental fate data and proposed testing**

Photodegradation in air was calculated to have a half-life of 8.3 hours for BISCEP monomer and 2.7 hours for BISCEP dimer with the AOP program. No data is available on the water stability of BISCEP. A biodegradation study showed that BISCEP is not biodegradable.

Level III fugacity modeling shows that BISCEP monomer and dimer distribute mainly to water and soil when discharged equally in air, water and soil.

**Therefore, an hydrolysis study according to OECD 111 test guideline is proposed to complete the environmental fate data.**

### **3) Review of existing ecotoxicity data and proposed testing**

Acute toxicity studies on fish, daphnia and algae are available. Algae was the most sensitive species (72h- ErC50 = 113 mg/l, 96h-ErC50 = 73.5 mg/l, 14d-NOEC = 18 mg/l). Acute toxicity values on fish (96h-LC50) and daphnia (48h-EC50) were above 100 mg/l.

No additional testing is proposed for purposes of the HPV program.

### **4) Review of existing toxicity data and proposed testing**

#### **a) Acute oral toxicity :**

The acute oral toxicity of BISCEP has been examined in two studies. These studies were not GLP studies (1977 and 1979 studies) but they followed the main requirements of the OECD guideline 401. Both studies were performed on rats, by gavage and the LD50 values were 580 and 810 mg/kg b.w.. The lowest value was selected. BISCEP is moderately toxic by the oral route. These data fulfill the SIDS endpoint for acute toxicity and no additional testing is proposed.

#### **b) Repeated dose toxicity**

BISCEP was tested in a 90-day study in male and female rats. The product was administered orally by gavage at dose levels of 0, 100, 200 and 500 mg/kg bw/day. The predominant toxic effect of the oral administration of the test material was dose-related clonic convulsions in the 200 and 500 mg/kg dose groups. No treatment related toxic change was seen in the 100 mg/kg dose group except some convulsive activity. No NOEL or NOAEL was established for this study.

This study was considered as valid with restrictions as some requirements of the OECD guideline 408 were not followed (urea and creatinin were not measured in blood analyses, uterus, thymus and spleen were not weighted at necropsy and the administered volumes were low as no vehicle was used). However the results are considered as reliable.

Moreover, BISCEP was also studied in a 21-day dermal study on rabbits. Significant lesions which appeared to be related to the test material were present in the liver, kidneys and treated skin. These lesions were of low severity and probably represented mild reversible injury.

This study was considered as valid with restrictions: the main reason was that, due to the oily nature of the test material, it was impossible to clean all the material from the rabbit after the designated daily exposure period. Therefore all high dose group rabbits were entirely contaminated and this contamination decreased with the dose in the other treated groups. Therefore it is difficult to discern whether the observed effects are purely due to dermal exposure or a combination of dermal and oral exposure due to preening. However this study provides valuable information on dermal repeated dose toxicity.

These studies fill the SIDS endpoint for repeated toxicity.

c) Genotoxicity : Gene mutation

BISCEP was negative in a bacterial gene mutation assay with *Saccharomyces cerevisiae* (strain D4) and *Salmonella typhimurium* (strains TA1535, TA1537, TA1538, TA98 and TA100). This study was considered as valid with restrictions as the test was not repeated, the plates were not done in triplicates and the solvent was not indicated.

BISCEP was also negative in another bacterial gene mutation assay (Ames test) with *Salmonella typhimurium* (strains TA1535, TA1537, TA1538, TA98 and TA100). This study was considered as valid with restrictions as the test was not repeated.

BISCEP was negative in a mammalian cell gene mutation assay conducted with L5178Y Mouse lymphoma cells TK <sup>+</sup>/<sub>-</sub>. This study was GLP. It was considered as valid with restrictions as the test was not repeated, the results mention data on historical controls that are not available and some cultures at low dose levels were lost.

BISCEP was also negative in another mammalian cell gene mutation assay conducted with L5178Y Mouse lymphoma cells TK <sup>+</sup>/<sub>-</sub>. This study was considered as valid with restrictions as the test was not repeated.

All these studies were conducted both with and without metabolic activation.

Although these studies show some deficiencies (mentioned above), collectively they provided consistent negative results both in bacterial and mammalian systems. Thus they satisfy the requirement for gene mutation data.

d) Genotoxicity : Chromosomal aberration

No data available for BISCEP. A test is proposed. Following recommendations of the USEPA and in the best interests of animal welfare, an *in vitro* mammalian chromosome aberration test according to OECD Test Guidelines 473 rather than an *in vivo* mammalian erythrocyte micronucleus study is proposed.

e) Reproduction toxicity

No data available for BISCEP. No test is proposed. Following recommendations of the USEPA, considering the adequacy of the developmental toxicity study and of the evaluation of reproductive organs in the 13-week repeated-dose toxicity study, no reproduction toxicity test is deemed necessary.

Moreover, in a two generation reproduction study conducted on the downstream product (phosphonic acid, 2-chloroethyl-), no effects were observed on fertility, gestation, mating, organ weights or histopathology in any generation (MRID 41508701, EPA 738-R-95-003, April 1995).

f) Developmental toxicity

A teratology study was conducted with BISCEP on rats and did not reveal any teratogenic effect. The NOAEL for developmental effects was 600 mg/kg bw/day (highest dose tested) and the NOAEL for maternal effects was 400 mg/kg bw/day (some deaths occurred at 600 mg/kg). The results are considered reliable and this study fills the SIDS endpoint

g) Conclusion for the toxicology testing

- The SIDS endpoints on acute toxicity, repeated toxicity, genotoxicity as regards gene mutation and developmental toxicity are filled adequately.

- A mammalian erythrocyte micronucleus study according to the OECD guideline 473 is proposed to fill the SIDS endpoint on genotoxicity as regards chromosome aberration.
- No specific toxicity study has been performed to fill the SIDS endpoint on reproduction toxicity. The information issued from developmental toxicity study and evaluation of reproductive organs in the 13-week repeated-dose toxicity study was judged sufficient to prevent testing.

An additional publication (in Russian) (1) has been reviewed. This document and the primary references which are cited in this document don't provide any reliable information to address any SIDS endpoint.

## **V) SUMMARY**

In summary, the testing proposed in Table 2 will complete the data acquisition requirements for BISCEP under the U.S. Environmental Protection Agency High Production Volume (HPV) Chemical Challenge Program.

## **VI) ROBUST STUDY SUMMARIES**

An IUCLID Data Set for BISCEP is appended.

## **VII) REFERENCE**

(1) Chura, D. A.; Kuz'minov, B. P.; Galushka, A. I.; Nektegaev, I. A.; Kokot, V. R.; Grushka, O. I.; Karatsincheva, M. Yu. Toxicological characterization of bis-(2-chloroethyl)2-chloroethylphosphonate; Original Title: Toksikologicheskaya kharakteristika bis-(2-khloretil)2-khloretilfosfonata, Meditsina Truda i Promyshlennaya Ekologiya, (1993) (2) 21-2. (published in Russian)

**Table 1 : Significant Data on BISCEP**

<b>BISCEP</b>		
<b>Endpoint</b>	<b>Result</b>	<b>Comment</b>
<b>Physicochemical</b>		
Melting point	28 °C	Rhodia MSDS
Boiling point	170-172 °C at 6.7 hPa	Handbook (BISCEP monomer)
Density	1.41 g/cm <sup>3</sup> at 25°C	Rhodia MSDS
Vapor pressure	1.47 x 10 <sup>-4</sup> hPa at 25°C 2.75 x 10 <sup>-8</sup> hPa at 25°C	Estimation for BISCEP monomer Estimation for BISCEP dimer
Log Pow	1.65 at 25°C 1.47 at 25°C	Estimation for BISCEP monomer Estimation for BISCEP dimer
Water solubility	1.5 % w/w at 20°C	Rhodia MSDS
<b>Environmental fate and pathway</b>		
Photodegradation	DT50 in air = 8.3 h DT50 in air = 2.7 h	Estimation for BISCEP monomer Estimation for BISCEP dimer
Stability in water	No data	-
Transport/distribution	Air = negligible Water = 51.4% Soil = 48.6% Sediment = 0.03% Air = negligible Water = 56.5% Soil = 43.5% Sediment = 0.03%	Fugacity model level III (BISCEP monomer)  Fugacity model level III (BISCEP dimer)
Biodegradation	Not biodegradable	Experimental result
<b>Ecotoxicity</b>		
Acute fish	96h-LC50 > 100 mg/l	Experimental result
Acute daphnia	48h-EC50 = 240 mg/l	Experimental result
Algae	96h-ErC50 = 73.5 mg/l 14d-NOEC = 18 mg/l	Experimental result
<b>Toxicology</b>		
Acute toxicity	oral LD50 = 580 +/- 90.1 mg/kg bw Rat (M&F)	The lowest LD50 among the reliable results.
Repeated dose toxicity	90-day oral study in rats (M&F) : Predominant effects following oral administration were clonic convulsions (observed from the lowest dose of 100 mg/kg bw/day onwards).  21-day dermal study in rats (M&F). Lesions probably reversible and of low severity in the liver, kidneys and skin (lowest dose tested : 1000 mg/kg bw).	Experimental result  Experimental result
Genetic toxicity <i>in vitro</i> Gene mutation	Ames tests : negative Mouse lymphoma tests : negative	Experimental results. Experimental results.
Chromosomal aberration	No data.	
Genetic toxicity <i>In vivo</i>	No data.	
Toxicity to reproduction	No data.	
Developmental tox/teratogenicity	Teratology study in rats : negative	Experimental result

*BISCEP monomer : main component (CAS No 6294-34-4)*

*BISCEP dimer : other main component (noted as main impurity in IUCLID) (CAS No 58823-09-9)*

**Table 2 : Availability of data and proposed testing on BISCEP**

<b>BISCEP</b>							
<b>Endpoint</b>	<b>Available</b>	<b>GLP</b>	<b>OECD study</b>	<b>Other study</b>	<b>Estim. method</b>	<b>Acceptable</b>	<b>SIDS testing required</b>
<b>Physicochemical</b>							
Melting point	Y	N	N	Y	-	Y	N
Boiling point	Y	N	N	Y	-	Y	N
Density	Y	N	N	Y	-	Y	N
Vapor pressure	Y	N	N	N	Y	Y	N
Oct:water partition coef	Y	N	N	N	Y	Y	N
Water solubility	Y	N	N	Y	-	Y	N
<b>Environmental fate and pathway</b>							
Photodegradation	Y	N	N	N	Y	Y	N
Stability in water	N	-	-	-	-	-	Y
Transport/distribution	Y	N	N	N	Y	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
<b>Ecotoxicity</b>							
Acute fish	Y	N	N	Y	N	Y	N
Acute daphnia	Y	N	N	Y	N	Y	N
Algae	Y	N	N	Y	N	Y	N
<b>Toxicology</b>							
Acute toxicity	Y	N	N	Y	N	Y	N
Repeated dose toxicity	Y	Y	N	Y	N	Y	N
Genetic toxicity: Gene mutation							
- Bacterial (Ames test)	Y	N	N	Y	N	Y	N
- Mammalian (L5178Y Mouse lymphoma test)	Y	Y	N	Y	N	Y	N
Chromosome aberrat.	N	-	-	-	-	-	Y
Toxicity to reproduction	N	-	-	-	-	-	N*
Devel. tox/teratogen.	Y	Y	N	Y	N	Y	N

Y : yes

N : no

\* : see details in chapter e) of section IV.4